

## SYNTHESIS AND CHARACTERIZATION OF RELATED SUBSTANCES OF RUPATADINE FUMARATE: AN ANTIHISTAMINE DRUG

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### ABSTRACT

Rupatadine fumarate is a potent dual antagonist of histamine and platelet-activating factor antagonist (PAF antagonist) and used to treat allergies. During the process development of rupertadine fumarate, five related substances were observed in Rupertadine fumarate and one new related compound was observed during the stress oxidative degradation study. These related substances were identified as process related impurities, starting material related impurity and oxidative degradation related impurity. Present work describes the synthesis and characterization of all these impurities.

### KEYWORDS

1. Rupertadine fumarate, 2. Antihistamine, 3. Related substance, 4. Quaternization, 5. Anti-allergic

### INTRODUCTION

Rupertadine fumarate <sup>i-iii</sup> possesses a potent PAF antagonist and antihistamine activity and has been selected from a series of N-alkyl pyridine derivatives that has demonstrated a potent dual antihistamine and PAF antagonist activity in animal and human models <sup>iii</sup>. The efficacy of rupertadine for the treatment of allergic rhinitis (both intermittent and persistent) and chronic idiopathic urticaria has been well established in several controlled clinical studies <sup>iv</sup>.

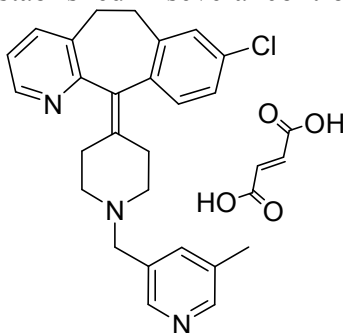


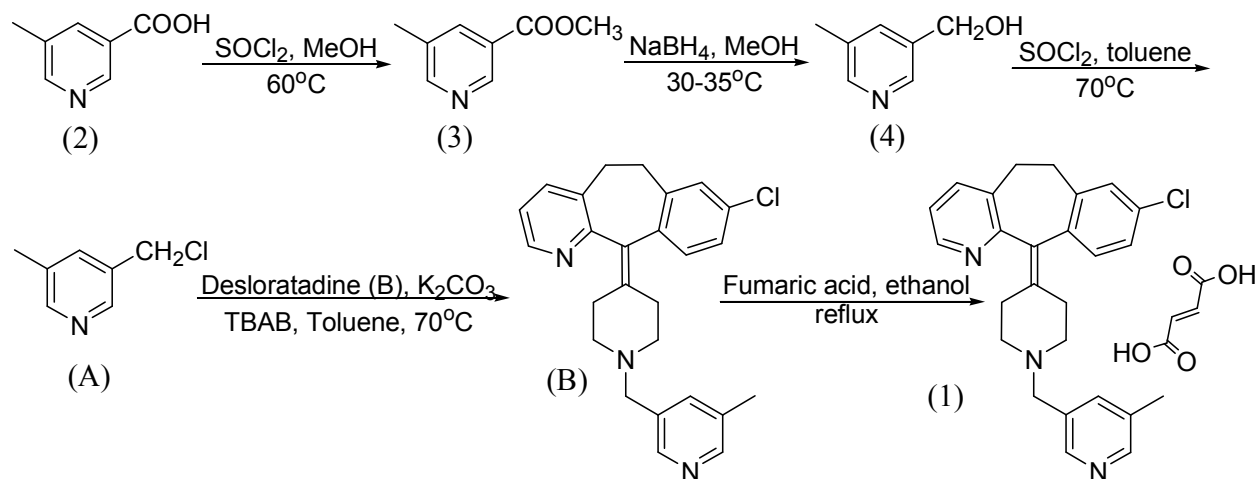
Fig (1)

Rupatadine (Fig. 1) acts as a long-acting, non-sedative antagonist at histaminergic H1-receptors. Rupatadine has been shown to have higher affinity for the H1-receptor than fexofenadine and Levocetirizine<sup>v</sup>. Rupatadine has other anti-allergic properties<sup>vi</sup> such as inhibition deregulation, induced by the immunological and non-immunological stimulants and the inhibition of release of cytokines, particularly the tumor necrosis factor alpha (TNF-alpha) in human mastocytes and monocytes<sup>vii</sup>. In vitro metabolism studies indicate that Rupatadine is metabolized mainly by the cytochrome P-450 in liver<sup>viii</sup>.

The presence of related substances as impurities in an active pharmaceutical ingredient (API) can have a significant impact on the quality and safety of the drug products. Hence, it is necessary to study the impurity profile of any API and its control during the manufacturing of the drug substance. In order to meet the stringent regulatory requirement, any impurity, which is formed at a level of  $\geq 0.10\%$  with respect to the API, should be identified and controlled as per ICH guidelines<sup>ix</sup>. Although study on some related compounds of Rupatadine Fumarate and their identification using HPLC and Mass analysis has been reported in literature<sup>x-xiv</sup>. Analytical impurity profiling of rupatadine fumarate prepared following scheme 1 has been studied by Trivedi et al<sup>xv</sup>. Herein, we wish to discuss synthesis, identification and characterization of the impurities related to Rupatadine fumarate.

## RESULTS AND DISCUSSION

Rupatadine fumarate **1** is designated chemically as 8-Chloro-6,11-dihydro-11-[1-[(5-methyl-3-pyridinyl) methyl] -4-piperidinylidene] -5Hbenzo [5,6] cyclohepta [1,2-b] pyridine fumarate. The synthesis of rupatadine (Scheme 1) involves the esterification of 5-methyl-3-nicotinic acid **2** to get methyl 5-methyl-3-nicotinate **3** on reduction with sodium borohydride in methanol yields 5-methyl-3-pyridylmethanol **4**. On chlorination of **4** in toluene gives 5-methyl-3-chloromethyl pyridine **A**. N-alkylation of **A** with desloratadine **B** in toluene in the presence of the base gives rupatadine base **5**. This was converted to fumarate salt using fumaric acid<sup>xvi-xvii</sup>.



Scheme 1 : Synthetic scheme of Rupatadine Fumarate **1**

Rupatadine fumarate prepared following scheme 1, contains five major impurities in crude material and out of that four impurities were present in rupatadine fumarate. Impurity **A** and **B** were unreacted intermediates, confirmed by HPLC analysis. While compound **C** and **D** were process related impurities confirmed by LC/MS analysis. Furthermore we have analyzed the

mother liquor and found impurity **E**. During forced degradation study rupatadine has shown significant tolerance toward acid and base stress conditions. In oxidative degradation condition, one major degradation substances observed on HPLC. Mass analysis revealed that it is having mass 433 ( $M + 1$ ).

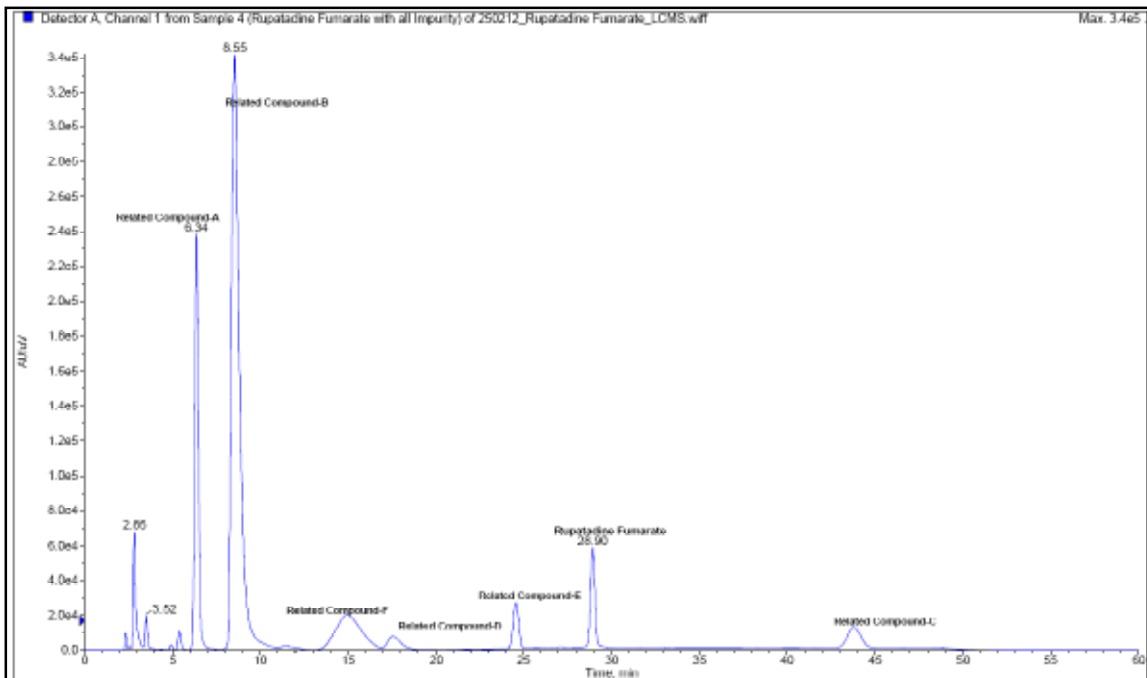
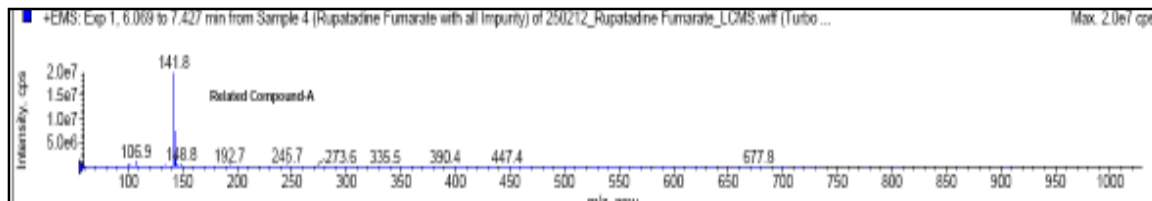
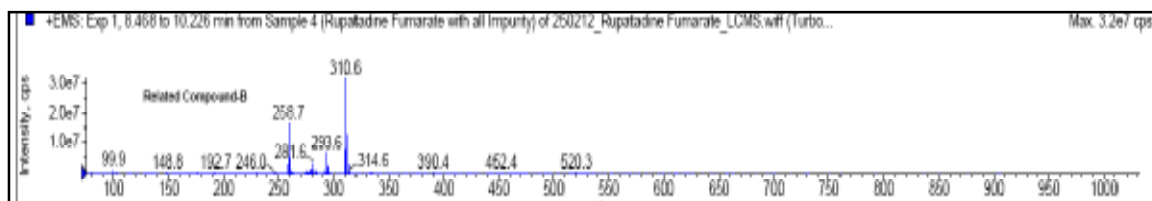


Fig 2: Typical chromatogram of Rupatadine fumarate with Ms/MS Impurities

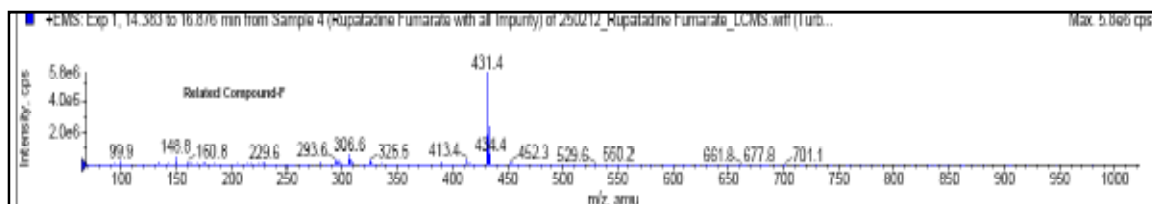
(a). MS/MS spectrum of Related compound A



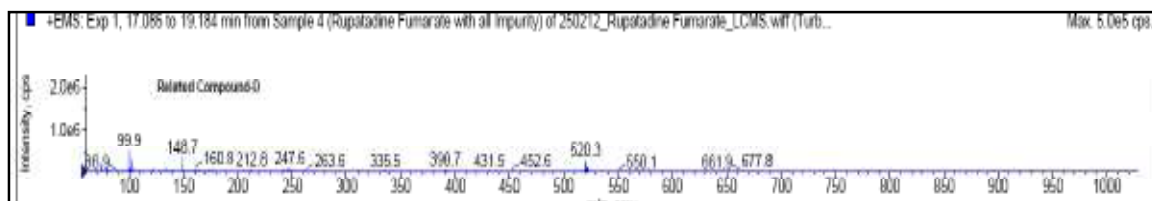
(b) MS/MS spectrum of Related compound B



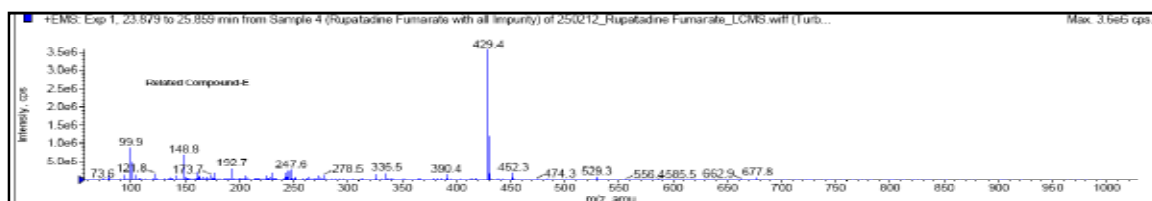
(c) MS/MS spectrum of Related compound C



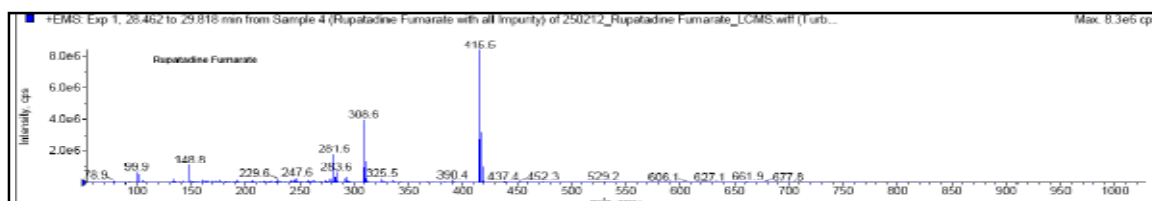
(d) MS/MS spectrum of Related compound D



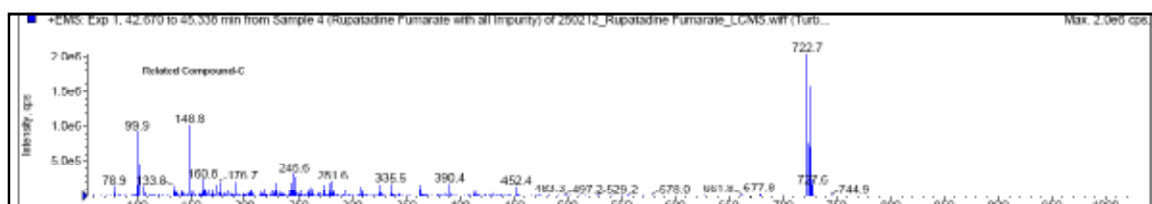
(e) MS/MS spectrum of Related compound E



(f) MS/MS spectrum of Related compound (F)



(g) MS/MS spectrum of RUPATADINE FUMARATE G



To establish profiles of rupatadine fumarate and related substances, we have further decided to synthesis and characterize the impurity present in rupatadine fumarate. Impurity **A** and **B** are well established intermediates for rupatadine synthesis (Scheme 1), so we have spiked these intermediate with crude sample on HPLC and established as impurity, **A** and **B** which were named as 5-methyl-3-chloromethyl pyridine and desloratadine respectively.

During the impurity profile study one related substance shows mass at 724 ( $M + 1$ ). Which is a dimeric product of rupatadine<sup>x</sup>. The starting material 5-methyl-3-nicotinic acid **2** contains ~0.2%

3,5-pyridine dicarboxylate as an impurity, which on subsequent convert into disaster, diol and finally Dichloro compound which further reacts with desloratadine and form impurity **C**.

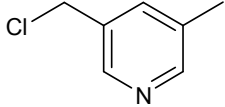
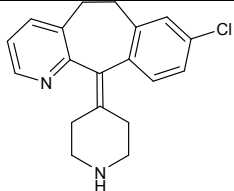
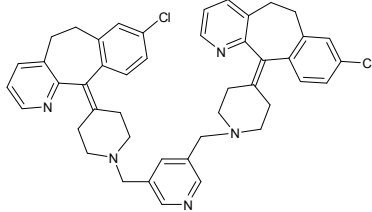
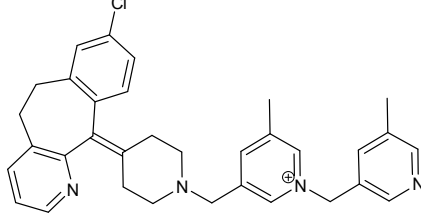
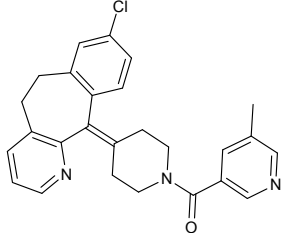
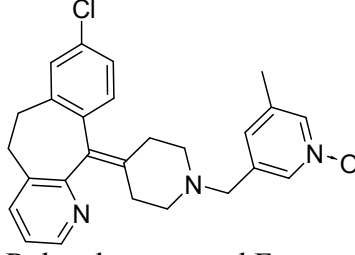
LC/MS analysis confirms that one of the related substance was 523 (M + 1), this shows one additional lutidine moiety in rupatadine structure. During reaction that impurity was forming ~2% for complete characterization of this impurity we have subjected mother liquor for column chromatography, but failed to isolate the pure compound due to the unstable nature of compound in silica gel. Finally we have decided to synthesize the impurity and various solvents were explored to Quaternization the rupatadine using 5-methyl-3-chloromethyl pyridine **A** to prepare impurity in sizable amount and we have found out that impurity level in the reaction using DMF solvent was quite high ~23%. After subjecting crude material for column chromatography followed by preparative TLC we have isolated pure compound and characterized as Quaternary impurity **D** and its presence confirmed by HPLC.

On analysis of mother liquor shows a major impurity and its molecular mass was 430 (M + 1). This shows additional oxygen in rupatadine molecule. This is amide derivative of desloratadine with compound **2** and characterized as impurity **E**, which was synthesized as depicted in reaction scheme 2 in and its presence confirmed by HPLC<sup>xi-xiv</sup>. This impurity was forming by reaction of acid chloride of compound **2** with desloratadine because a small amount of compound **2** is present as a impurity with compound **4** at stage 3 in scheme 1.

During forced degradation study we have found out major degradation about 5% in oxidative stress condition. We repeated the same condition at higher scale to prepare oxidative degradation product. After isolation of crude material, it was further purified using column chromatography and degradation product was identified as impurity **F**.

Impurity **D** and impurity **F** are new impurities and first time synthesize and characterized. Impurity **C** and impurity **E** are known in literature<sup>x-xiv</sup>. Synthetic methodology for impurity **E** is well documented<sup>i,ii,xi-xiv</sup>. While synthesis and characterization of impurity **C** is first time reported.

**Table 1 : Related substances of Rupatadine Fumarate**

S. No.	Structure	Name	Source
1	 <p>Related compound A</p>	3-chloromethyl-5-methyl pyridine	Intermediate
2	 <p>Related compound B</p>	Desloratadine	Key starting material
3	 <p>Related compound C</p>	Diamine compound	Process impurity
4	 <p>Related compound D</p>	Quaternary compound	Process impurity
5	 <p>Related compound E</p>	Amide compound	Process impurity
6	 <p>Related compound F</p>	N-oxide compound	Oxidative degradation impurity

## EXPERIMENTAL

**Material and methods:** All the solvents and reagents were used of commercial grade and used without further purification. Reaction progress was monitored on TLC and HPLC wherever required.

### High Performance Liquid Chromatography

Samples were analyzed on Dionex Ultimate 3000 separation module equipped with Ultimate 3000 PDA Detector (Dionex Ltd., Germany) using a Waters Symmetry C8 (250mm X 4.6mm), 5 $\mu$ m, Waters Corporation, Ireland). Mobile phase-A consisted of a, 0.02M ammonium acetate adjusted pH 6.0  $\pm$  0.05 using diluted glacial acetic acid solutions- and Mobile Phase –B- Methanol with a timed gradient mode T (min) /%B: 0/60, 15/60, 25/80, 45/80, 50/60, 60/60. A flow rate of 1.0mL/min was throughout the analysis. The injection volume was 20 $\mu$ L for sample concentration of 1.0mg/mL prepared in the diluent (mixture of Water: Methanol in the volume ratio 50: 50v/v). Detector wavelength was fixed at 264nm and the column oven temperature was maintained at 40°C.

### Liquid Chromatography-Tandem mass spectrometry (LC/MS/MS)

The MS and MS/MS studies were performed on 3000 Q-Trap mass spectrometer (AB Sciex, Foster City, CA, USA). The instrument was operated in an enhanced production mode in positive polarity mode with the following setting: Collision energy of 40V, Collision energy spread 10V and declustering potential 20V. Nitrogen was used as a curtain gas at a pressure of 25psi, and as collision associated dissociations (CAD) gas. Zero air is used as Nebulizer gas and heater gas at a pressure of 60 psi. The ion spray voltage was 4500V. The HPLC consisted of a LC10AD binary gradient pump, a SPD-10AVP UV detector, SIL-10HTC auto sampler and a Column Oven CTO-10ASVP (Shimadzu Corporation, Kyoto, Japan). A Waters Symmetry C8 (250mm X 4.6mm), 5 $\mu$ m, Waters Corporation, Ireland). Mobile phase-A consisted of a, 0.02M ammonium acetate adjusted pH 6.0  $\pm$  0.05 using diluted glacial acetic acid solutions- and Mobile Phase –B- Methanol with a timed gradient mode T (min) /%B: 0/60, 15/60, 25/80, 45/80, 50/60, 60/60. A flow rate of 1.0mL/min was throughout the analysis. The injection volume was 20 $\mu$ L for sample concentration of 1.0mg/mL prepared in diluents (mixture of Water: Methanol in the volume ratio 50: 50v/v). Detector wavelength was fixed at 264nm and the column oven temperature was maintained at 40°C.

### NMR spectroscopy

<sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at 300.13MHz using a Bruker Ultra shield 300MHz spectrometer (Bruker, Fallanden, Switzerland) equipped with a 5mm BBO probe and a z-gradient shim system. The <sup>1</sup>H spectra were recorded with 1s pulse repetition time using 30 flip angle, while <sup>13</sup>C spectra were recorded with power gated decoupling using 30° dimethyl sulphoxide-d<sub>6</sub>. The <sup>1</sup>H and <sup>13</sup>C chemical shift values were reported on the  $\delta$  scale in ppm relative to DMSO-d<sub>6</sub> (2.50ppm). All spectra were recorded with sample spinning.

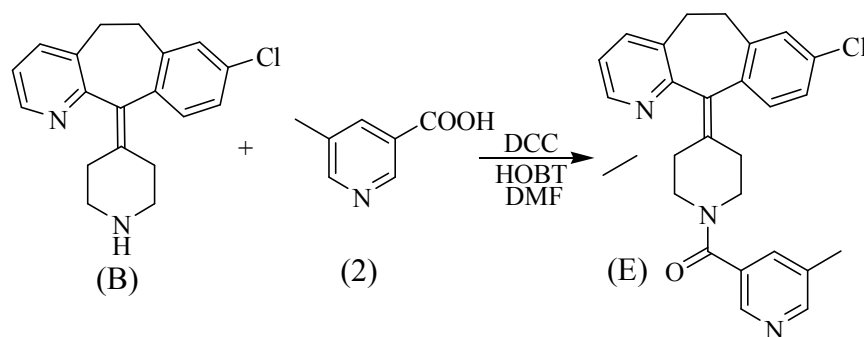
### FT-IR spectroscopy

The IR spectrum was recorded in the solid state as KBr pellet using Shimadzu FTIR-8400 (Shimadzu Corporation, Kyoto, Japan) with a DTGBS KBr detector. Data were collected between 400 and 4000cm<sup>-1</sup>, with a resolution of 4.0cm<sup>-1</sup>. A total of 16 scans was obtained and processed using the IR solution software version 1.5.

### Synthesis of amide impurity (E)<sup>xi-xiv</sup>

Desloratadine (10 g, 0.03mol), 5-methyl nicotinic acid (4.4 g, 0.03mol) and 1-hydroxy benzotriazole (0.43 g, 0.003mol) were dissolved in 120 mL of DMF at 25-30°C. A solution of DCC (12 g, 0.06mol) in DMF (40 mL) was added slowly to above solution and the reaction

mixture was stirred at 25-30 °C for 3-4 h. Reaction was monitored using TLC, the reaction mixture was cool to 5-10 °C and filtered. The solvent was distil out completely at 60-70°C under reduced pressure. Toluene (40mL) was added and reaction mass was further cool to 5-10°C. By-product was filtered and toluene layer was washed with an aqueous sodium hydroxide solution (4.8g NaOH in 60 mL water), followed by water washing. Toluene was removed under reduced pressure at 65-70°C and residue was further purified by column chromatography and pure amide impurity E was isolated. (8.3 g, yield 60%, purity by HPLC 99.7%); m.p. 143-146 °C; FT IR (KBr): 765,1610, 3340  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  2.0 (s, 2H), 2.37 (s, 3H), 2.4-2.58 (m, 2H), 2.78-2.92 (m, 2H), 3.2-3.4 (m, 4H), 3.6 (m, 1H), 4.2 (m, 1H), 7.1-7.2 (m, 4H), 7.44-7.45 (m, 1H), 7.58 (s, 1H), 8.45-8.47 (m, 2H); MS m/z (EI): 430.2 (M + 1).



Scheme 2 : Synthetic scheme of Amide impurity **E**

### Synthesis of diamine impurity (C) [10]

#### Pyridine-3, 5-dicarboxylic acid dimethyl ester **7**

To a suspension of pyridine 3,5-dicarboxylic acid (20 g, 0.12 mol) in methanol (100 mL), slowly added thionyl chloride (26 mL, 0.36 mol). The reaction mixture was heated to 65-70°C and maintained for 3-4 h. After completion of the reaction on TLC, methanol was distil out and oily residue was diluted with dichloromethane. The reaction mixture was basifying using ammonia (20 mL) and the organic layer was separated. Dichloromethane was removed under reduced pressure to obtain the title compound **7** as oily mass (15 g, yield 64%, purity by HPLC 97.5%). FT IR (KBr): 1103, 1720  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  3.98 (s, 6H), 8.7 (s, 1H), 9.2 (s, 2H); MS m/z (EI): 196 (M + 1).

#### (5-hydroxymethyl-pyridine-3-yl)-methanol **8**

Compound **7** (15 g, 0.08 mol) was dissolved in THF (75 mL) and solution was added to a suspension of LAH (7.2 g) in THF (75 mL) at -70 to -75°C. Stirred reaction mixture at -70 to -75°C for 30-45 min. Ethyl acetate (40 mL) was slowly added followed by addition of saturated ammonium chloride solution (40 mL). Warmed reaction mixture 5-10°C and inorganic material was filtered. The solvent was distil out and the residue was diluted with dichloromethane (60 mL) and water (150 mL) at 25-30°C. pH is adjusted 2-3 using concentrate HCl and the aqueous layer was separated and the dichloromethane layer was discarded. Further dichloromethane (120 mL) was added in aqueous layer and pH 9-10 of reaction mass was adjusted using 20% sodium hydroxide solution. The aqueous layer was separated and the dichloromethane layer was discarded. The aqueous layer was distill off under reduced pressure at 55-60°C to obtain the title compound **8** as a green oily mass. (7 g, yield 65%, purity by HPLC 84.8 %). FT IR (KBr): 3300-3500  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  4.7 (s, 4H), 7.9 (s, 1H), 8.4 (s, 2H); MS m/z (EI): 140 (M + 1).

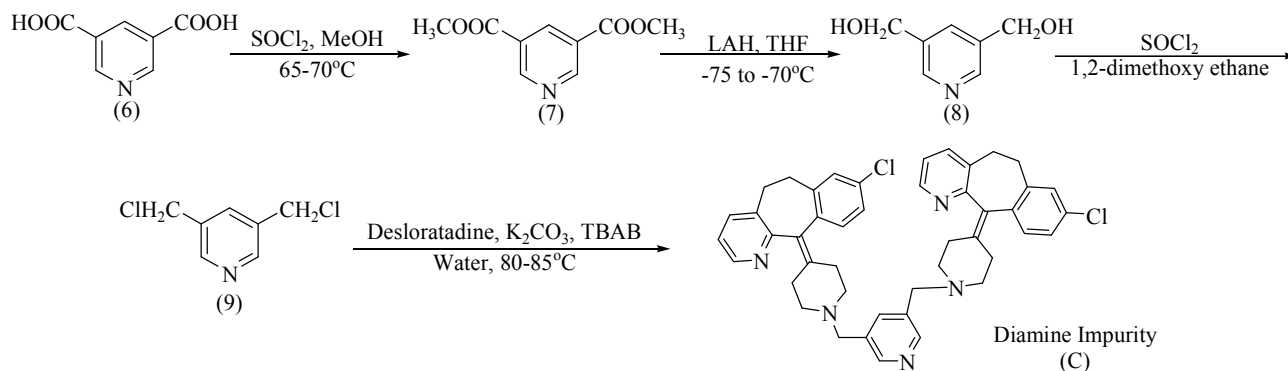


### 3, 5-bis-chloromethyl-pyridine 9

To a solution of compound 8 (7 g, 0.05 mol) in 1, 2-dimethoxy ethane (35 mL) thionyl chloride (6.5 mL, 0.09 mol) was slowly added at room temperature. The reaction mixture was heated to reflux at 80-85°C for 2 h. After completion of the reaction on TLC, the solvent was distilled out under reduced pressure to obtain the title compound 9 as a yellow solid (10.1 g, yield 95 %, purity by HPLC 95.5%). FT IR (KBr): 1275 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, CD3OD) δ 3.3 (s, 2H), 3.5 (s, 2H), 8.8 (s, 1H), 9.0 (s, 2H); MS m/z (EI): 178 (M + 1).

### Synthesis of diamine impurity (C)

Compound 9 and desloratadine 3 (29 g, 0.09 mol) was dissolved in toluene (120 mL). Potassium carbonate (35 g, 0.25 mol) and water (60 mL) were added. Reaction mass was heated to 80-85°C and stirred for 3-4 h. Reaction mass was cooled to 25-30°C. The organic layer was separated and washed with 15% potassium dihydrogen phosphate solution followed by 20% sodium chloride solution. The solvent was removed under reduced pressure to obtain the title compound C as a pale yellow solid (10 g, yield 67.7%, purity by HPLC 93%); m.p. 140-142 °C; FT IR (KBr) : 1363, 1477, 1583 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO d<sub>6</sub>) δ 2.11-2.16 (m, 8H), 2.28-2.31 (m, 6H), 2.56 (m, 4H), 2.74-2.8 (m, 4H), 3.2-3.29 (m, 4H), 3.47 (s, 4H), 7.01-7.03 (d, J= 8.1 Hz, 2H), 7.18-7.21 (d, J= 8.4 Hz, 2H), 7.25-7.26 (d, J = 2.1 Hz, 2H), 7.52-7.54 (d, J = 6.6 Hz, 2H), 7.58 (s, 1H), 8.29-8.30 (d, J = 3.9 Hz, 2H), 8.33-8.34 (d, J = 1.5 Hz, 2H); MS m/z (EI): 723.9 (M + 1).

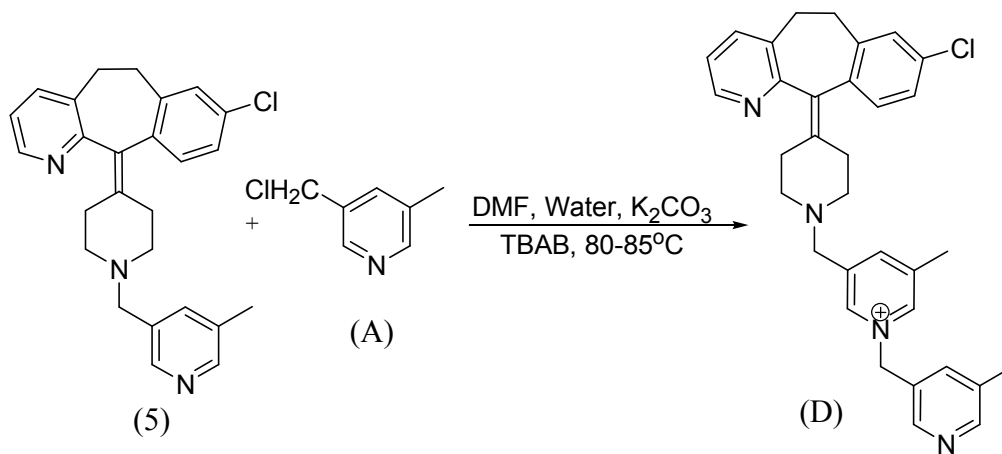


Scheme 3 : Synthetic scheme of Diamine impurity C

### Synthesis of Quaternary impurity (D)

Rupatadine free base (2.9 g, 0.007 mol) and compound 5 (1.9 g, 0.01 mol) was dissolved in DMF (12mL) followed by the addition of potassium carbonate (3.5 g, 0.025 mol), TBAB (0.2 g) and water (1 mL). The reaction mixture was heated to 80-85°C and stirred for 3-4 h. Impurity formation was monitored on HPLC till impurity level reaches up to 23%. The reaction mixture was cooled to 25-30°C and filtered to remove the inorganic material. The solvent was removed from the filtrate under reduced pressure at 65-70°C to obtain a residue. The residue was further purified using preparative TLC (mobile phase 5% methanol in MDC) to obtain compound D as a semi solid (0.7 g, yield 20%; purity by HPLC 91.6%); IR (KBr): 1369, 1473, 1560 cm<sup>-1</sup>; <sup>1</sup>H NMR (300 MHz, DMSO-d<sub>6</sub>) 1.2 (s, 1H), 2.0-2.3 (m, 10H), 2.4 (s, 4H), 2.7-2.8 (m, 2H), 3.2-3.4 (m, 2H), 3.7 (s, 2H), 5.9 (s, 2H), 7.0 (d, J = 8.1 Hz, 1H), 7.1-7.2 (m, 2H), 7.3 (s, 1H), 7.5-7.6 (d, J = 7.5, 1H), 7.9 (s, 1H), 8.2-8.3 (d, J = 3.6, 1H), 8.3-8.4 (d, J = 11.1 Hz, 2H), 8.7 (s, 1H), 9.2 (s, 1H), 9.3 (s, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>): 17.743, 17.858, 30.251, 30.376, 30.528, 31.036, 53.786, 53.938, 57.354, 60.276, 122.323, 125.600, 128.943, 129.844, 130.729, 131.493, 132.409, 133.217,

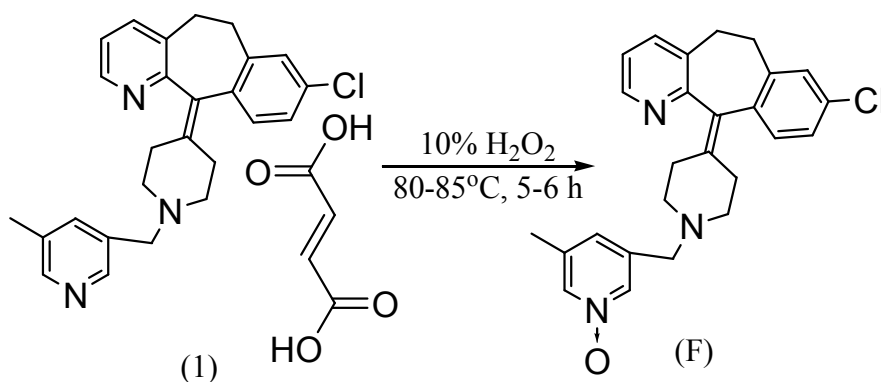
133.360, 136.951, 137.239, 137.365, 137.852, 138.806, 139.181, 140.105, 141.671, 143.122, 146.226, 146.304, 147.136, 150.757, 157.011; MS m/z (EI): 522.9 (M + 1).



Scheme 4 : Synthetic scheme of Quaternary impurity **D**

### Synthesis of N-oxide impurity (F)

To a solution of 10% hydrogen peroxide (500 mL), rupatadine fumarate (3g, 0.006mol) was added at 25-30 °C and heated to 80-85 °C for 5-6 h. The reaction mixture was cooled to 20-25 °C and extracted with dichloromethane (2 X 100 mL). Dried the organic layer and distil out atmospherically. The residue was further purified by column chromatography to obtain compound **F** (0.8 g, yield 33%; purity by HPLC 91.58%); m.p. 75-80°C; IR (KBr): 1363, 1477, 1585  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR (300 MHz, DMSO  $d_6$ )  $\delta$  2.3-2.35 (s, 3H), 2.43-2.55 (m, 2H), 2.64-2.85 (m, 4H), 2.88-3.69 (m, 7H), 4.54 (m, 2H), 4.79 (s, 4H), 5.29 (s, 1H), 7.09-7.25 (m, 4H), 7.43-7.45 (m, 1H), 7.85-7.88 (m, 1H), 8.33-8.51(m, 3H); MS m/z (EI): 431.9 (M + 1).



Scheme 5 : Synthetic scheme of N-oxide impurity **F**

### CONCLUSION

In Present paper a detailed study of the related compounds of rupatadine fumarate has been carried out. The entire related compound has been synthesized and well characterized. Related compounds **D** and **F** are novel compound and first time synthesise.

## ACKNOWLEDGEMENT

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